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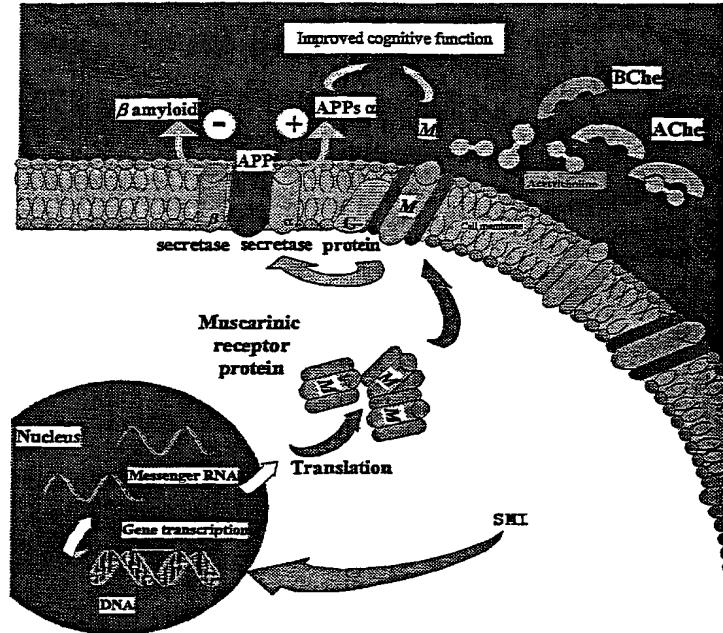
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(54) Title: 5-HYDROXYSAPOGENIN DERIVATIVES WITH ANTI-DEMENTIA ACTIVITY



WO 01/23408 A1

(57) Abstract: The invention discloses the use of 5-hydroxysapogenin derivatives in the treatment of cognitive dysfunction and similar conditions. Methods of treatment and pharmaceutical composition are also disclosed.



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5-HYDROXYSAPOGENIN DERIVATIVES WITH ANTI-DEMENTIA ACTIVITY

5 The present invention relates to sapogenin derivatives and their use in
treating cognitive dysfunction and allied conditions; and to compositions for use in
such treatments. The invention is also concerned with the treatment of conditions
that are characterised by a deficiency in the number or function of membrane-
bound receptors. In the following, the present invention will be described
10 principally with reference to the treatment of Alzheimer's disease (AD) and senile
dementia of the Alzheimer's type (SDAT), where deficiencies in a number of
receptor types have been demonstrated. However, it is to be understood that the
present invention relates generally to the treatment of conditions attributable to
intrinsic pathological conditions and/or exposure to adverse environmental
15 conditions these conditions being characterised by a deficiency in the number or
function of membrane-bound receptors or a deficiency in transmission at the
junctions between neurones or at the junctions of neurones and effector cells.

20 Conditions of the type mentioned above include Parkinson's disease, Lewi-
body dementia, postural hypotension, autism, chronic fatigue syndrome,
Myasthenia Gravis, Lambert Eaton disease, diseases and problems associated with
25 Gulf War Syndrome, occupational exposure to organophosphorus compounds and
problems associated with ageing.

25 Alzheimer's disease (AD) and senile dementia of the Alzheimer's type
(SDAT) are grave and growing problems in all societies where, because of an
increase in life expectancy and control of adventitious disease, the demographic
profile is increasingly extending towards a more aged population. Agents which
can treat, or help in the management of, AD/SDAT are urgently required.

30 Age-associated memory impairment (AAMI) is a characteristic of older
patients who, while being psychologically and physically normal, complain of
memory loss. It is a poorly defined syndrome, but agents which are effective in
treatment of AD/SDAT may also be of value in these patients.

35 Research into AD/SDAT is being carried out by traditional and
conventional medical research methods and disciplines. In conventional medicine,

- 2 -

there are several approaches to the treatment of AD/SDAT. It is known that the biochemical processes subserving memory in the cerebral cortex are (at least in part) cholinergically-mediated. Those skilled in the art will know that "cholinergically mediated" mechanisms may be directly attributable to acetylcholine acting on receptors, and these are direct effects. Other, clinically useful effects may also be caused by modulation of release of acetylcholine from pre-synaptic nerve endings or inhibition of enzymes that destroy acetylcholine. These modulating factors may be exerted through neurones where the mediator is non-cholinergic; these are referred to as indirect effects. Some attempts at treatment have focussed on the role of other mediators such as 5-hydroxytryptamine, which is a mediator in other areas of brain, such as the mid-brain nuclei. However, since fibres from these areas are projected forward into the cerebral cortex where the primary transmitter is acetylcholine, attention has focussed on the management of this mediator in the search for appropriate therapeutic agents.

Cholinergic strategies for the treatment of AD/SDAT have been directed at several points along the pathway of formation, synaptic release and removal of released acetylcholine.

One approach involves treatment with high doses of lecithin and other precursors of acetylcholine. This is of limited use in producing sustained improvements in cognitive performance.

Another approach involves the use of vegetable drugs such as *Polygalae* root extract, which has been shown to enhance choline-acetylcholine transferase (CAT) activity and nerve growth factor (NGF) secretion in brain. Oral administration of NGF has no effect on central nervous system neurons because it is a high molecular weight protein that cannot pass through the blood-brain barrier. However, agents which can pass through the blood-brain barrier and have a stimulating effect on NGF synthesis in the central nervous system have been proposed for the improvement of memory-related behaviour.

The results of a third clinical approach, which uses cholinesterase inhibitors such as tacrine hydrochloride, have been marginally more positive than the above. Substances obtained from plants used in Chinese and Western medicine, for

- 3 -

example huperzine, galanthamine, and physostigmine have all been shown to be of some - although limited - benefit in the treatment of AD/SDAT in clinical studies and also in laboratory models. All of these substances are inhibitors of acetylcholine esterase (AChE). In patients with AD/SDAT, there may be reduced synthesis of acetylcholine (ACh), reduced efficiency in release of ACh from presynaptic stores, and a decrease in the number or function of postsynaptic (M₁) receptors. Reductions in pre-synaptic M₂ receptors have also been shown. The beneficial effect of AChE inhibitors is attributed to enhancement of acetylcholine levels at synapses in brain by slowing down the destruction of released transmitter.

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Compositions which modulate cholinergic function are known to affect memory and recall. For example, nicotine stimulates nicotinic acetylcholine receptors, and the short lived memory enhancing effects of cigarette smoking are thought to be due to the effect of nicotine. Scopolamine, an antagonist of acetylcholine, will produce amnesia and impaired cognitive function manifesting in psychomotor tests as a prolongation of simple reaction times, possibly as a result of impaired attention, and is used for this purpose as an adjunctive analgesic treatment. The amnesic effect of scopolamine can be antagonised by nicotine.

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There are two families of nicotinic receptor subtypes (α and β), and each includes four subgroups which differ in ligand specificity. The role of nicotinic receptors in the CNS is not well understood at the molecular level. It is possible that agents binding to nicotinic receptors may modify the rate of turnover at muscarinic receptor sites in brain. Nicotinic receptors are ligand-gated ion channels, and their activation causes a rapid (millisecond) increase in cellular permeability to Na⁺ and Ca⁺⁺, depolarisation and excitation.

30

Another class of cholinergic receptors can be stimulated by muscarine. Such muscarinic (M) receptors are G protein-coupled receptors. Responses of muscarinic receptors are slower; they may be excitatory or inhibitory. They are not necessarily linked to changes in ion permeability. Five types of muscarinic receptors have been detected by cholinergic receptor cloning, and are designated as m₁-m₅. Pharmacological effects are associated with four of the cloned receptors and they are designated as M₁-M₄ based on pharmacological specificity.

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Using specific receptor proteins and monoclonal antibodies, it has been

possible to further localise muscarinic receptors in brain as m_1 (postsynaptic) and m_2 (presynaptic). In heart, M_2 receptors are postsynaptic. Presynaptic muscarinic receptors are thought to be inhibitory, the binding of ACh to these receptors attenuating the release of further ACh to provide a negative feedback mechanism for Ach release. Selective M_2 receptor antagonists which are preferentially distributed to the brain may therefore be useful in treating Alzheimer's disease.

It is known that, in disease states such as AD/SDAT, there is general neuronal loss and deficits in cholinergic nerve function. It has been speculated that the high affinity nicotinic binding sites in the remaining cholinergic neurons might be converted to low affinity binding sites in treating such diseases, thereby sustaining transmitter release. By lowering the affinity of the nicotinic binding sites, a quick desensitising process is avoided.

Agonist activation at nicotinic receptors in brain has rapid onset and offset. A decreased affinity of the nicotinic receptors will reduce the desensitisation process. Schwarz R.D. et al (J. Neuro Chem 42, (1984), 1495-8) have shown that nicotine binding sites are presynaptically located on cholinergic (and also 5-hydroxytryptaminergic and catecholaminergic) axon terminals. A change in high affinity binding sites on AD/SDAT may also induce a change in the modulatory effect the nicotinic binding sites may have on other transmitter systems.

Presynaptic cholinergic mechanisms are also under inhibitory control by GABAergic neurons and this inhibition is thought to be intensified in AD/SDAT. Removal or reduction of this inhibition intensifies presynaptic cortical cholinergic activity and enhances cognitive processing.

The interactions of interneuronal fibres innervated by nicotine (reducing binding affinity), and dis-inhibition of GABAergic fibres both have a presynaptic locus.

This is a simplistic model of central transmission, but provides a framework for understanding the attempts which have been made to increase the effective concentration of acetylcholine in central synapses. This further illustrates the concept of direct and indirect action. There are disadvantages attaching to the three conventional therapeutic approaches to AD/SDAT treatment mentioned above:

- 5 -

5 ACh precursor supplementation, agonist replacement and acetylcholine esterase inhibition. These treatments may result in a short-term increase in the availability of ACh which may activate feedback mechanisms resulting in the desensitisation of postsynaptic receptors. On theoretical grounds, long term benefits would not be predicted and when treatment is interrupted, any benefits in management of AD/SDAT and AAMI disappear and the condition may even be aggravated.

10 It has been shown that a compound with M_1 agonist and M_2/M_3 antagonist activity improved cognitive performance in SDAT patients (Sramak et al, Life Sciences vol. 2, No. 3, 195-202, 1997). However, this compound causes unacceptable cholinergic side effects, such as fatigue, diarrhoea and nausea.

15 A more radical approach to AD/SDAT and AAMI aims to increase the number of postsynaptic (M_1) receptors, in brain. It is known from Chinese Patent No. CN1096031A, that sarsasapogenin (SaG) can up-regulate M_1 cholinergic receptors.

20 Patent applications have been published which claim the usefulness of a number of steroid sapogenins having spirostane, furo-spirostane, spirosolane or solanidine structures in the treatment of diseases including SDAT. Two patent publications are of particular relevance here: Chinese patent publication No CN1096031A claims the use of the spirostane sapogenin, sarsasapogenin, in the treatment of SDAT. The disclosure in this document, however, is brief. The other document of relevance is patent publication DE 4303214A1 which claims the use 25 of a very wide range of saponins and sapogenins in the treatment of a whole range of diseases that the inventors consider to be of viral origin. This disclosure is however of dubious value in that it is well recognised that there is no infective element to a very large number of the conditions that are characterised by deficient synaptic transmission and thus the basic premise of the alleged invention is flawed. In addition they present no data of any kind that allows one skilled in the art to be 30 able select a preferred compound from the large number that are claimed.

35 The inventors have found that certain sapogenin derivatives exhibit the ability to regulate receptors. In particular, these compounds have been found to increase the number of M_2 receptors in the brain. Thus, according to one aspect of the invention, there is provided the use of a sapogenin derivative of general

- 6 -

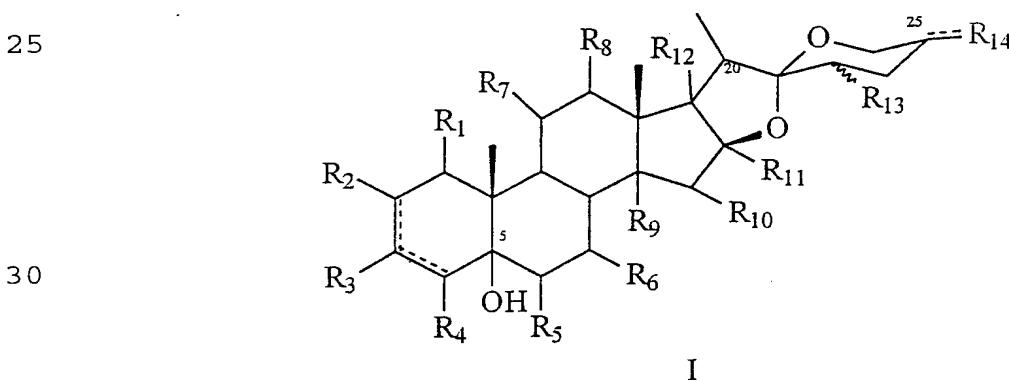
formula (I) in the manufacture of a medicament for the treatment of a condition characterised by a deficiency in membrane-bound receptor number or function.

5 Those skilled in the art will be aware of the relationship between saponins and their sapogenins, and that the latter tend to be fat-soluble whereas the saponins tend to be water-soluble. Sapogenins are therefore better able to cross the blood-brain barrier. The skilled man will also be aware of the epimerisation of certain sapogenins under conditions of acid hydrolysis.

10 The variation in pharmacological properties and pharmacodynamic actions of various types of sapogenins underlines the need for selection of those agents which are most useful in the treatment of A/SDAT. The discovery of novel facts about the action of sapogenin derivatives has made it possible to determine which substances are most useful for the treatment of AD/SDAT and the like.

15 The inventors have found that the above-described properties are exhibited by sapogenin derivatives wherein there is a hydroxy at C5 of the fused ring system.

20 Accordingly, the sapogenin derivatives of interest in this invention have the following general formula (I):



35 their stereoisomers racemic mixtures, their pharmaceutically acceptable pro-drugs

- 7 -

and salts.

In the general Formula (I):

- R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈, R₁₀, are, independently of each other, either H, OH, =O, and OR where R = optionally substituted alkyl, optionally substituted acyl, optionally substituted carbamoyl, alkoxy carbonyl;
- R₉, R₁₂, R₁₁, R₁₃ can be either a H, OH, OR where R = optionally substituted alkyl, optionally substituted acyl, optionally substituted carbamoyl, alkoxy carbonyl;
- R₁₄ = optionally substituted alkyl group
- represents an optional double bond,

and the stereochemistry at C5 can be either R or S

15 Preferably, in the general formula (I):

- R₄, R₉, R₁₂, R₁₃ = H
- R₁, R₂, R₃, R₅, R₆, R₇, R₈, R₁₀, can be independently of each other either H, OH, =O, OR where R = optionally substituted alkyl, optionally substituted acyl, optionally substituted carbamoyl, alkoxy carbonyl;
- R₁₁ = H, OH, OR where R = optionally substituted alkyl, optionally substituted acyl, optionally substituted carbamoyl, alkoxy carbonyl;
- R₁₄ = optionally substituted alkyl group
- represents an optional double bond,

and the stereochemistry at C5 can be either R or S

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More preferably, in the formula (I):

- R₁ = R₂ = R₄ = R₆ = R₇ = R₈ = R₁₀ = R₁₁ = R₉ = R₁₂ = R₁₃ = H,
- R₃ = OH, or OCOCH₃, or =O
- R₅ = OH, or OCOCH₃, or =O
- R₁₄ = CH₃
- represents an optional double bond,

and the stereochemistry at C5 can be either R or S

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As used hereabove and hereafter:

- 8 -

"Acyl" means an H-CO- or Alkyl-CO- group wherein the alkyl group is as herein described. Preferred acyls contain a lower alkyl. Exemplary acyl groups include formyl, acetyl, propanoyl, 2-methylpropanoyl, butanoyl and palmitoyl.

5 "Alkyl" means an aliphatic hydrocarbon group which may be straight or branched having about 1 to about 20 carbon atoms in the chain. Preferred alkyl groups have 1 to about 12 carbon atoms in the chain. Branched means that one or more lower alkyl groups such as methyl, ethyl or propyl are attached to a linear alkyl chain. "Lower alkyl" means about 1 to about 4 carbon atoms in the chain which may be 10 straight or branched. Exemplary alkyl groups include methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *t*-butyl, *n*-pentyl, 3-pentyl.

15 "Optionally substituted" means that the said group may be substituted with one or more substituents which may be the same or different, and include halo, alkyl, cycloalkyl, hydroxy, alkoxy, amino, acylamino, aryl, arylamino, carboxy, alkoxycarbonyl, aralkoxycarbonyl, heteroaralkoxycarbonyl, optionally substituted carbamoyl.

20 The term "pharmaceutical composition" means a composition comprising a compound of formula I and at least one component selected from the group comprising pharmaceutically acceptable carriers, diluents, adjuvants, excipients, or vehicles, such as preserving agents, fillers, disintegrating agents, wetting agents, emulsifying agents, suspending agents, sweetening agents, flavoring agents, perfuming agents, antibacterial agents, antifungal agents, lubricating agents and 25 dispensing agents, depending on the nature of the mode of administration and dosage forms.

30 "Pharmaceutically acceptable" means it is, within the scope of sound medical judgement, suitable for use in contact with the cells of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio.

35 "Pharmaceutically acceptable dosage forms" means dosage forms of the compound of the invention, and includes, for example, tablets, dragees, powders, elixirs, syrups, liquid preparations, including suspensions, sprays, inhalants tablets, lozenges, emulsions, solutions, granules, capsules and suppositories, as well as

liquid preparations for injections, including liposome preparations. Techniques and formulations generally may be found in Remington, Pharmaceutical Sciences, Mack Publishing Co., Easton, PA, latest edition.

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"Pharmaceutically acceptable prodrugs" as used herein means those prodrugs of the compounds useful according to the present invention which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals with undue toxicity, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio, and effective for their intended use, as well as the zwitterionic forms, where possible, of the compounds of the invention. The term "prodrug" means compounds that are rapidly transformed *in vivo* to yield the parent compound of the above formula, for example by hydrolysis in blood. Functional groups which may be rapidly transformed, by metabolic cleavage, *in vivo* form a class of groups reactive with the carboxyl group of the compounds of this invention. Because of the ease with which the metabolically cleavable groups of the compounds useful according to this invention are cleaved *in vivo*, the compounds bearing such groups act as prodrugs. A thorough discussion of prodrugs is provided in the following: Design of Prodrugs, H. Bundgaard, ed., Elsevier, 1985; Methods in Enzymology, K. Widder et al, Ed., Academic Press, 42, p.309-396, 1985; A Textbook of Drug Design and Development, Krosgaard-Larsen and H. Bundgaard, ed., Chapter 5; Design and Applications of Prodrugs p.113-191, 1991; Advanced Drug Delivery Reviews, H. Bundgard, 8, p.1-38, 1992; Journal of Pharmaceutical Sciences, 77, p. 285, 1988; Chem. Pharm. Bull., N. Nakaya et al, 32, p. 692, 1984; Pro-drugs as Novel Delivery Systems, T. Higuchi and V. Stella, Vol. 14 of the A.C.S. Symposium Series, and Bioreversible Carriers in Drug Design, Edward B. Roche, ed., American Pharmaceutical Association and Pergamon Press, 1987, which are incorporated herein by reference.

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"Pharmaceutically acceptable salts" means the relatively non-toxic, inorganic and organic acid addition salts, and base addition salts, of compounds of the present invention. These salts can be prepared *in situ* during the final isolation and purification of the compounds. In particular, acid addition salts can be prepared by separately reacting the purified compound in its free base form with a suitable organic or inorganic acid and isolating the salt thus formed. See, for

- 10 -

example S. M. Berge, et al., *Pharmaceutical Salts*, *J. Pharm. Sci.*, 66: p.1-19 (1977) which is incorporated herein by reference. Base addition salts can also be prepared by separately reacting the purified compound in its acid form with a suitable organic or inorganic base and isolating the salt thus formed. Base addition salts include pharmaceutically acceptable metal and amine salts.

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Sapogenin derivatives of interest in the present invention may occur naturally in a range of plant species, notably from the genera *Smilax*, *Asparagus*, *Anemarrhena*, *Yucca* and *Agave*. The species presently of greatest interest include *Smilax regelii* Kilip & Morton - commonly known as Honduran sarsaparilla; *Smilax aristolochiaefolia* Miller - commonly known as Mexican sarsaparilla; *Smilax ornata* Hooker - commonly known as Jamaican sarsaparilla; *Smilax aspera* - commonly known as Spanish sarsaparilla; *Smilax glabra* Roxburgh; *Smilax febrifuga* - Kunth - commonly known as Ecuadorian or Peruvian sarsaparilla; *Anemarrhena asphodeloides* Bunge; *Yucca schidigera* Roezl ex Ortgies; and *Yucca brevifolia* Engelm.

Sapogenin derivatives which may be of interest may also occur naturally in other genera, for example *Dioscorea*, *Trillium*, *Solanum*, *Strophanthus*, *Digitalis* and *Trigonella*. However, some sapogenin derivatives from these sources possess undesirable properties and are thus not recommended for use in the invention.

Sapogenin derivatives of the invention may also be commercially available; suppliers are well-known from the one skilled in the art and may include Sigma Aldrich, Research Plus Inc., Steraloids Inc., etc...

According to a further aspect of the invention, there is provided a process of preparation of the compounds of the invention.

Substituted sapogenins of the present invention may be prepared by synthetic methods. For instance, they may be prepared from unsubstituted sapogenin derivatives, which may occur naturally or be commercially available, as stated above.

Starting from these unsubstituted sapogenins, the reaction may involve at least one substitution step, wherein the functional group is substituted on the sapogenin derivative; usually, the starting product is an unsubstituted sapogenin having the required stereochemistry, and the reaction may involve the substitution of one OH-group by the functional radical desired; smilagenin and epismilagenin are

preferred as starting products.

Compounds useful according to the invention may be prepared by the application or adaptation of known methods, by which is meant methods used heretofore or described in the literature, for example those described by R. C. 5 Larock in *Comprehensive Organic Transformations*, VCH publishers, 1989.

In the reactions described hereinafter it may be necessary to protect reactive functional groups, for example hydroxy or carboxy groups, where these are desired in the final product, to avoid their unwanted participation in the reactions.

Conventional protecting groups may be used in accordance with standard practice, 10 for examples see T.W. Green and P.G.M. Wuts in *"Protective Groups in Organic Chemistry"* John Wiley and Sons, 1991; J. F. W. McOmie in *"Protective Groups in Organic Chemistry"* Plenum Press, 1973.

The compound thus prepared may be recovered from the reaction mixture by conventional means. For example, the compounds may be recovered by 15 distilling off the solvent from the reaction mixture or, if necessary after distilling off the solvent from the reaction mixture, pouring the residue into water followed by extraction with a water-immiscible organic solvent and distilling off the solvent from the extract. Additionally, the product can, if desired, be further purified by various well techniques, such as recrystallization, reprecipitation or the various 20 chromatography techniques, notably column chromatography or preparative thin layer chromatography.

According to a further aspect of the present invention, there is provided a pharmaceutical composition having cognitive function enhancing properties which 25 comprises an effective amount of a sapogenin derivative of the invention.

In a still further aspect, the sapogenin derivatives of the present invention are steroidal; they are preferably non-oestrogenic in effect.

In another aspect, the invention provides a pharmaceutical composition 30 having cognitive function enhancing properties which comprises an effective amount of a sapogenin derivative of the invention in the form of an extract derived from a plant of the genus *Smilax*, *Asparagus*, *Anemarrhena*, *Yucca* or *Agave*.

35 It will be appreciated that the invention embraces within its scope the use of

the compositions defined above. Thus, according to a fifth aspect, the present invention provides a method of enhancing cognitive function which comprises administering to a human or animal an effective dosage of a composition of the invention.

5 The invention also provides a method of enhancing cognitive function in a human or non-human animal, which comprises administering an effective dose of sapogenin derivatives of the invention. Also, it concerns the use of the sapogenin derivatives of the invention in food product or beverage for enhancing cognitive function.

10 As used herein, the term "cognitive function" refers to functions such as thinking, reasoning, remembering, imagining and learning.

According to a further aspect, the invention also relates to composition having cognitive function enhancing properties which comprises at least two, preferably two, sapogenin derivatives of the invention.

15 In identifying compounds that would have use in the treatment of SDAT and other diseases characterised by reductions in receptor numbers or synaptic transmission, the inventors have given consideration to the need to identify compounds that would have the desired effect but would be devoid of any oestrogenic effects, as these would be unacceptable, particularly in male patients. A number of the compounds claimed to have activity in patent application DE 4303214A1 have marked oestrogenic activity and are therefore unacceptable. Preferably, sapogenin derivatives of the present invention however, does not display oestrogenic activity. In addition these compound were tested at other steroid receptors and were found to have no activity at any of the following receptors:

Progesterone

Glucocorticoid

Testosterone

30 Sapogenin derivatives of the present invention have also been tested for activity in a number of in-vitro assays. The assays/experiments that were considered of key importance in determining possible activity in the elevation of membrane bound receptor numbers were as follows:

35 Chinese hamster ovary (CHO) cells transfected with the a DNA fragment coding for a muscarinic receptor. The cell line used for the majority of the

- 13 -

experiments was a cell line expressing the m2 receptor. The methods and the results of these experiments are now described in turn.

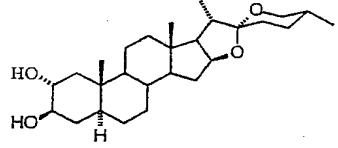
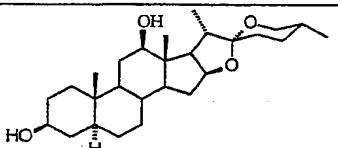
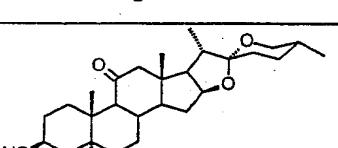
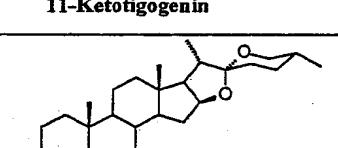
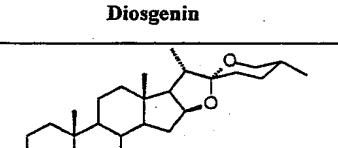
CHO cell line experiments

The effects of various compounds on the expression of m2 receptors on CHO cells transfected with DNA for the m2 receptor were investigated. Receptor numbers were assayed using tritiated QNB binding and subtracting non-specific binding. Compounds were dissolved in DMSO and DMSO was used as a control.

Compounds were tested at a range of final concentrations. Compounds were also tested in the presence and absence of tamoxifen to try to distinguish an oestrogen receptor mediated mechanism.

The results are summarised in the Table 1 below. The results are summarised in the Table 1 below. Compounds are active when the effect on receptor expression given as a percentage increase compared to control is more than 15%.

Table 1 Effects of sapogenin derivatives on the expression of m₂ receptors on CHO cells

Compound	Molar concentration	Activity	
	10^{-5}	Not active	
10		10^{-5}	Not active
15		10^{-5}	Not active
20		10^{-5}	Not active
25		10^{-5}	Not active

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Thus the experiments indicate that the saponin derivatives of the invention were able to increase the number of muscarinic receptors expressed on the surface of CHO cells cultured in-vitro. The effect was not antagonised by tamoxifen, indicating that the mechanism involved did not involve the oestrogen receptor.

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It appears from the experimental work conducted that the compounds of this

- 15 -

invention act to normalise muscarinic receptor number - i.e. they tend to prevent decline in receptor number with time, and also tend to restore receptor number to normal levels when given to cells in which the receptor number is depressed.

5 It is speculated here that the effect of the active compound claimed in this patent may operate through an effect on G protein and that the effects on receptor numbers are secondary to an effect on G-protein. When a membrane bound G-protein linked receptor is stimulated two basic sets of events are initiated: the effector response; and the internalisation of the receptor. The subsequent
10 processing of the receptor to the state where it is again in a form on the cell surface or other membrane surface where it can interact with another receptor ligand appears to be subject to a number of factors. A number of these factors or mechanisms appear to be G-protein linked. There is evidence that activation of m_1 receptors may have an effect on G-protein expression or levels. It is speculated that
15 the actions of the compounds described in this patent may be due to an interaction in the processes of receptor regeneration, G-protein linkage or G-protein homeostasis.

20 An alternative hypothesis is that the compounds are increasing the synthesis or release or a decreased rate of degradation of neurotropic factors such as brain derived growth factor and/or nerve growth factor. These effects on growth factors might be due to an effect of the compound on a cytosolic or nuclear receptor or the binding of a compound to a promoter region with a consequent effect directly on the rate of production of mRNA for the growth factor or as a consequence of
25 increasing the production of another material factor such as G-protein or finally the effects may be secondary to an effect on receptor or G-protein procession.

30 The increased expression and/or abnormal processing of the amyloid precursor protein (APP) is associated with the formation of amyloid plaques and cerebrovascular amyloid deposits which are the major morphological hallmarks of Alzheimer's disease. Of particular interest are the processes regulating the proteolytic cleavage of APP into amyloidogenic and nonamyloidogenic fragments. The cleavage of APP by the enzyme α -secretase within the β -amyloid sequence of the protein results in the formation of a non amyloidogenic C-Terminal fragment, and the soluble APP α fragment; this latter fragment has been shown to have
35 neurotropic and neuroprotective activity as well as to enhance memory in mice when injected intra-cerebro-ventrally (ICV). In contrast, processing of APP by β -

- 16 -

secretase exposes the N-terminus of β -amyloid which is released by γ -secretase cleavage at the variable C-terminus. The resulting β -amyloid peptides, which contain 39-43 amino acids, have been shown to be neurotoxic and to accumulate in plaques which interfere with inter-neurone connections.

5

A number of studies have shown that stimulation of the protein-kinase (PKC) linked muscarinic M_1 and M_3 receptors results in an increase in α -secretase activity. As a consequence processing of APP to APP α with its neuroprotective effects is increased. In parallel, processing of APP by β - and γ -secretase is decreased and there is a consequential reduction of β -amyloid. Other transmitters such as nerve growth factor (NGF) and brain derived neurotropic factor (BDNF) as well as bradykinin and vasopressin may have similar effects in increasing the proportion of APP processed to APP α . There may be a number of factors involved in the effects of NGF which may include binding of the factor to the tyrosine kinase receptor (TrkA) and the stimulation of phospholipase C γ with subsequent phosphorylation and activation of protein kinase C (PKC) and increase in relative activity of α -secretase.

20 Any treatment which increases activity of protein-kinase C selectively in brain might therefore be expected to be of use in the management of Alzheimer's disease. Until recently agonists selective at the M_1 receptor have not been available. Non-selective agonists would be expected to stimulate pre-synaptic M_2 receptors which cause negative feedback and hence would further severely impair muscarinic transmission. Selective agonists at the M_1 receptor are now becoming available

25 (talsaclidine) and such agents are under investigation for the treatment of AD.

There is however, a substantial risk that, as with the chronic administration of any receptor agonist, the clinical benefits seen will be severely limited in terms of the size of benefit by reducing receptor numbers or reducing sensitivity and in terms of side effects due to lack of receptor specificity. Thus compounds as described in this invention, which selectively increase muscarinic receptor numbers or function, 30 would be expected to be devoid of the problems seen with a muscarinic agonist and hence have particular utility. Indeed the benefits may be seen in three parts as follows.

35 1. A selective increase in M_1 receptor numbers leading to increased synaptic transmission. Chronic administration of a selective agonist will, at best, have no

- 17 -

adverse effect on transmission;

2. Secondary to the increased receptor numbers, an increase stimulation of PKC with a consequential increase in α -secretase activity, leading to:

5

2.1 A reduced production of β -amyloid and a consequent reduction of plaque formation and neuronal loss;

10 2.2 An increase in APP α and a consequent improvement in cerebral function as witnessed by an improvement in short and long term memory.

15 In order to illustrate the invention further by way of non-limiting example, reference will now be made to the accompanying drawings and to the Example

which follows; in the drawings:

FIGURE 1 illustrates the results obtained in Example 1 below;

20 FIGURE 2 illustrates a hypothetical mode of action for sapogenin derivatives;

25

Referring to Fig.2, a diagrammatic representation of the function of sapogenin derivatives of the invention is shown. It is believed that sapogenin derivatives act primarily on cell nuclei; the invention is not, however, limited to any particular mode of action. The observed increase in muscarinic receptor number consequential upon administration of sapogenin derivatives is interpreted as leading to increased expression of muscarinic receptor protein. The possible link between the secretases and β -amyloid protein formation (discussed above) is indicated in the drawing.

30

The following Example is provided to illustrate the invention in a non-limiting manner.

Example 1

35

In a CHO cell line expressing recombinant human muscarinic receptors *in vitro*,

- 18 -

the number of muscarinic receptors tends to decline with time. Sapogenin derivatives of the invention (1-10 μ M) incubated for 72 hours increase muscarinic receptor density.

5 **Methods:**

Effect of sapogenin derivatives of the invention on muscarinic receptor density in CHO cells expressing recombinant human muscarinic receptors.

10 Chinese hamster ovary (CHO) cells expressing high levels of receptor (~2.2 pmoles receptor/mg protein) were cultured in flasks (150 ml) for 24 hours before the start of the experiment. Vehicle (DMSO) and sapogenin derivatives (at 1 and 10 μ M) were added to the medium for 48 h. The culture medium was discarded, the cells scraped off and resuspended in Hanks solution, centrifuged and m-
15 receptor levels determined by incubating with [3 H]-QNB for 30 min followed by liquid scintillation counting. Protein levels were determined by a micro Lowry method.

Results:

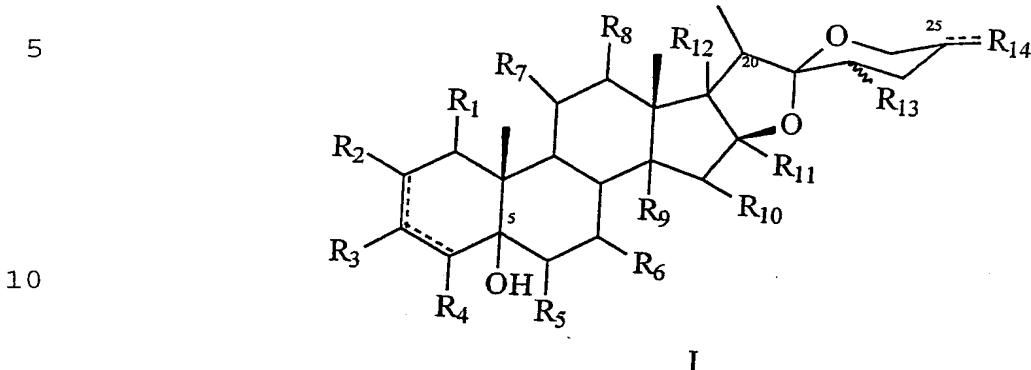
20 These are illustrated in Figure 1. Over the culturing period treatment with sapogenin derivatives of the invention prevents the decrease in muscarinic receptor number in a concentration-dependent manner.

25

- 19 -

CLAIMS:

1. Sapogenin derivatives of general formula I :



and their stereoisomers and racemic mixtures, their pharmaceutically acceptable
pro-drugs and salts,

wherein:

in the general formula (I):

20 - R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈, R₁₀, are, independently of each other, either H, OH, =O, and OR where R = optionally substituted alkyl, optionally substituted acyl, optionally substituted carbamoyl, alkoxy carbonyl;

- R₉, R₁₂, R₁₁, R₁₃ can be either a H, OH, OR where R = optionally substituted alkyl, optionally substituted acyl, optionally substituted carbamoyl, alkoxy carbonyl;

25 - R₁₄ = optionally substituted alkyl group

- represents an optional double bond,

and the stereochemistry at C5 can be either R or S.

30 2. Sapogenin derivatives according to claim 1, wherein in the general formula (I):

- R₄, R₉, R₁₂, R₁₃ = H

- R₁, R₂, R₃, R₅, R₆, R₇, R₈, R₁₀, can be independently of each other either H, OH, =O, OR where R = optionally substituted alkyl, optionally substituted acyl, optionally substituted carbamoyl, alkoxy carbonyl;

35 - R₁₁ = H, OH, OR where R = optionally substituted alkyl, optionally substituted

- 20 -

acyl, optionally substituted carbamoyl, alkoxy carbonyl;

- R_{14} = optionally substituted alkyl group

- represents an optional double bond,

and the stereochemistry at C5 can be either R or S.

5

3. Sapogenin derivatives according to any of the preceding claims, wherein in the general formula (I):

- $R_1 = R_2 = R_4 = R_6 = R_7 = R_8 = R_{10} = R_{11} = R_9 = R_{12} = R_{13} = H$,

- $R_3 = OH$, or $OCOCH_3$, or $=O$

10

- $R_5 = OH$, or $OCOCH_3$, or $=O$

- $R_{14} = CH_3$

- represents an optional double bond,

and the stereochemistry at C5 can be either R or S.

15

4. Use of the compounds as claimed in any preceding claim in the manufacture of a medicament for increasing the muscarinic receptor number or enhancing the function of muscarinic receptors in a human or non-human animal.

20

5. The use according to claim 4, wherein said medicament is for treating cognitive dysfunction.

6. The use according to claim 4 or 5, wherein said medicament is for enhancing cognitive function in a patient suffering from age-related cognitive dysfunction.

25

7. The use according to any of claim 4, 5 or 6, wherein said medicament is for treating a disease chosen from: Alzheimer's disease, senile dementia of the Alzheimer's type, Parkinson's disease, Lewy body dementia, postural hypotension, autism, chronic fatigue syndrome, Myasthenia Gravis, Lambert Eaton disease, diseases and problems associated with Gulf War Syndrome, occupational exposure to organophosphorus compounds and problems associated with ageing.

30

8. The use according to any of claim 4 to 7, wherein said medicament is for the treatment of a condition characterised by the presence of neurofibrillary tangles and/or β -amyloid plaques.

35

- 21 -

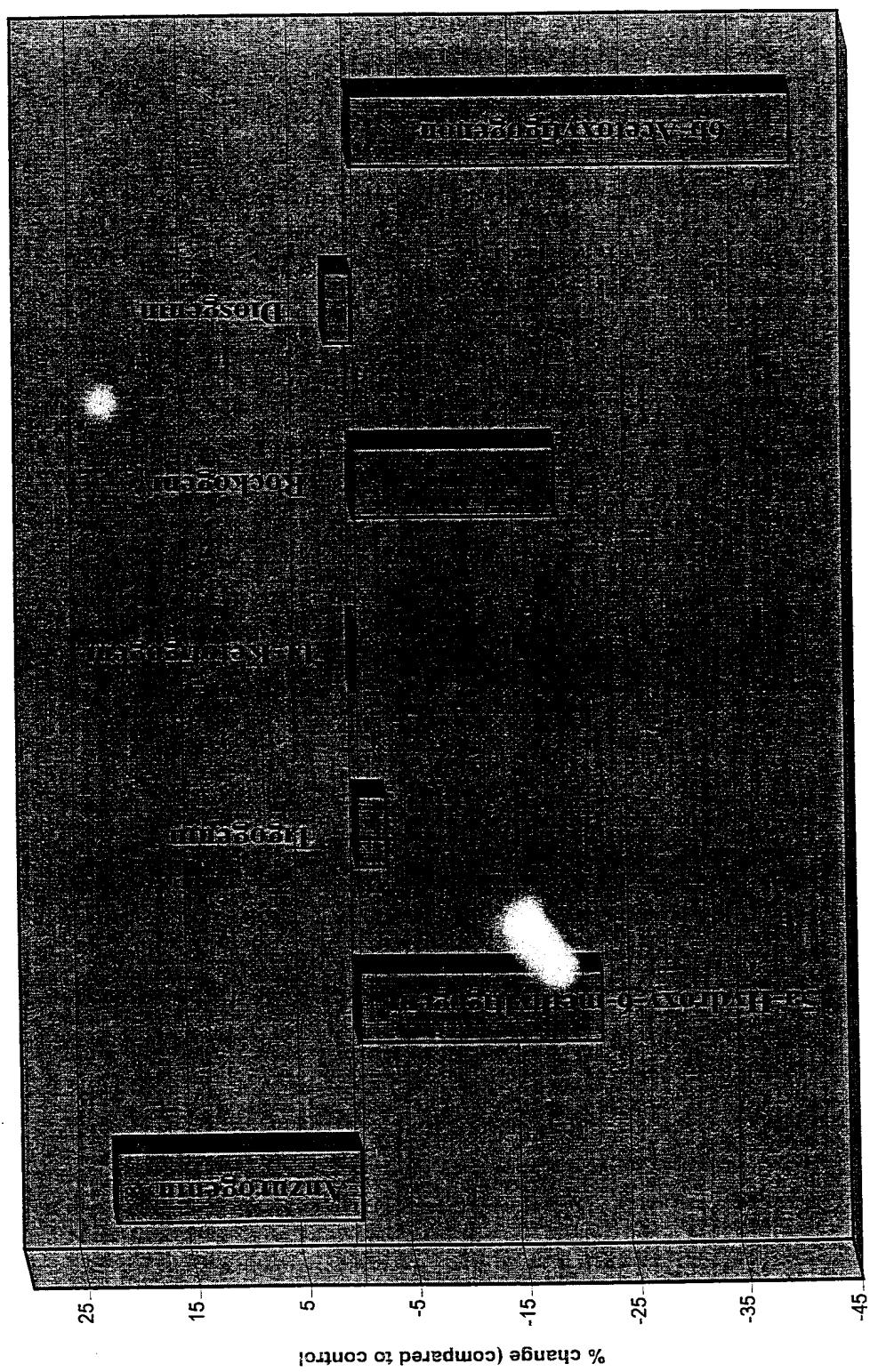
9. The use according to any of claim 4 to 8, wherein said medicament is for treating a disease selected from Alzheimer's disease, senile dementia of the Alzheimer's type.

5 10. A pharmaceutical composition having cognitive function enhancing properties which comprises a pharmacologically effective amount of a sapogenin derivative of formula (I) as defined in any of claims 1 to 3.

10 11. A pharmaceutical composition having cognitive function enhancing properties which comprises a pharmacologically effective amount of a sapogenin derivative of formula (I) as defined in any of claims 1 to 3 in the form of an extract derived from a plant of the genus Smilax, Asparagus, Anemarrhena, Yucca or Agave.

15 12. A non-therapeutic method of enhancing cognitive function in a human or non-human animal, which comprises administering an effective dose of a sapogenin derivative of formula (I) as defined in any of claims 1 to 4.

20 13. The use of a sapogenin derivative of formula (I) as defined in any of claims 1 to 4 in a food product or beverage to enhance cognitive function.



2 / 2

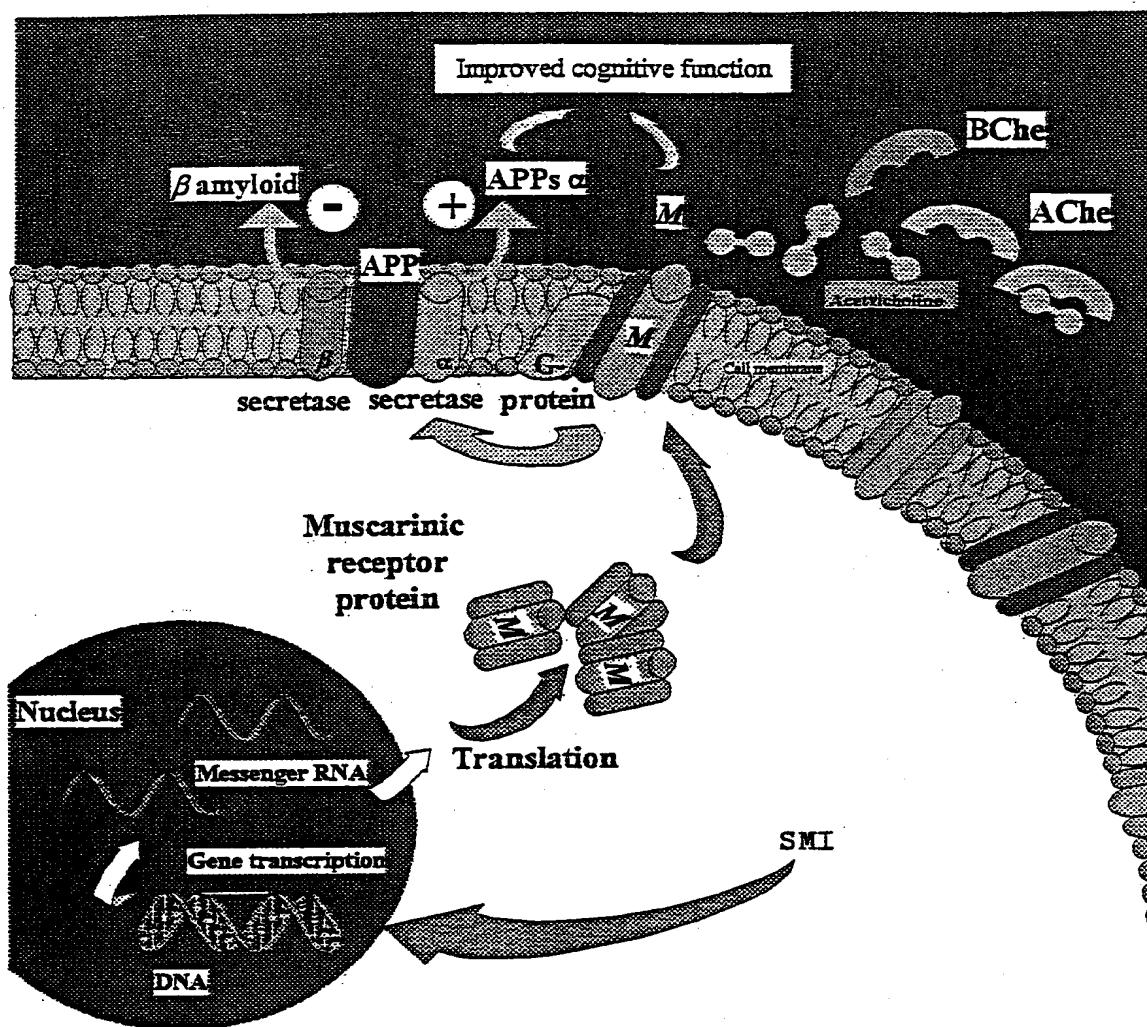


FIGURE 2

INTERNATIONAL SEARCH REPORT

Inte .ional Application No
PCT/GB 00/03750

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 7 C07J71/00 A61K31/58 A61P25/28 A61P25/14 A61P25/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 IPC 7 C07J A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, CHEM ABS Data, WPI Data, BEILSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 1 020 477 A (ALONSO BECERRA ESTHER MARIA ;COLL MANCHADO FRANCISCO (CU); JOMARRO) 19 July 2000 (2000-07-19) claim 1, compounds 2o, 2p and 2q ---	1-3
X	GB 852 847 A (BRITISH DRUG HOUSES LTD) 2 November 1960 (1960-11-02) example 36 ---	1-3 -/-

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority, claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

12 January 2001

30/01/2001

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Watchorn, P

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 00/03750

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DAWIDAR A M ET AL: "KETO-STEROIDS, I CONVERSION OF DIOSGENIN TO 6BETA-METHYL PREGN-4-ENE-6 ALPHA,20-DIOL-3,16-DIONE" ZEITSCHRIFT FUER NATURFORSCHUNG, TEIL B: ANORGANISCHE CHEMIE, ORGANISCHE CHEMIE, DE, VERLAG DER ZEITSCHRIFTEN FUER NATURFORSCHUNG,, vol. 37B, no. 7, 1 July 1982 (1982-07-01), pages 892-895, XP000614540 page 893; examples 2,7 ---	1-3
X	US 3 836 527 A (IRMSCHER K ET AL) 17 September 1974 (1974-09-17) example 2 ---	1-3
X	US 3 929 769 A (KAUFMANN HEINZ ET AL) 30 December 1975 (1975-12-30) example 13 ---	1-3
X	CHEMICAL ABSTRACTS, vol. 63, no. 13, 20 December 1965 (1965-12-20) Columbus, Ohio, US; abstract no. 18200b, M. TAKUCHI ET AL: "Steroids. XXVI. Isomeric 5.alpha.-pregnane-3,5,6,16,20-pentols and related compounds" XP002157202 abstract & CHEMICAL AND PHARMACEUTICAL BULLETIN., vol. 11, no. 1, 1963, pages 95-103, PHARMACEUTICAL SOCIETY OF JAPAN. TOKYO., JP ISSN: 0009-2363 ---	1-3
X	CHEMICAL ABSTRACTS, vol. 59, no. 11, 25 November 1963 (1963-11-25) Columbus, Ohio, US; abstract no. 12871g, C. WEI-SHAN ET AL: "Synthesis of C-6 methylene steroids" XP002157203 abstract & HUA HSUEH HSUEH PAO, vol. 28, no. 6, 1962, pages 394-397, ---	1-3
		-/-

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 00/03750

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>CHEMICAL ABSTRACTS, vol. 59, no. 5, 2 September 1963 (1963-09-02) Columbus, Ohio, US; abstract no. 5222a, HAYAO NAWA ET AL: "Steroids. XXVIII. Synthesis of polyhydroxy steroids. 4. Syntheses of pregnanetetrols" XP002157204 abstract & CHEMICAL AND PHARMACEUTICAL BULLETIN., vol. 11, 1963, pages 139-144, PHARMACEUTICAL SOCIETY OF JAPAN. TOKYO., JP ISSN: 0009-2363</p> <p>---</p>	1-3
X	<p>CHEMICAL ABSTRACTS, vol. 97, no. 7, 16 August 1982 (1982-08-16) Columbus, Ohio, US; abstract no. 49324, MIRONOVA, V. N. ET AL: "Hypocholesteremic action of phytocecdysones in rat experimental hypercholesterolemia" XP002157205 abstract & VOPR. MED. KHIM. (1982), 28(3), 101-5 ,</p> <p>---</p>	1,2,11
X	<p>DATABASE CHEMABS 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; 1995 YAO, XINSHENG ET AL: "identification of new constituents in Allium macrostemon Bunge and A. chinense G. Don bulbs and their anti-blood platelet agglutination activities" retrieved from STN Database accession no. 123:350230 XP002157206 abstract & CN 1 102 186 A (SHENYANG MEDICAL COLLEGE, PEOP. REP. CHINA) 3 May 1995 (1995-05-03)</p> <p>---</p>	1,11
A	<p>EP 1 024 146 A (INST OF RADIATION MEDICINE ACA) 2 August 2000 (2000-08-02) page 20, paragraph 46 page 21, compounds Va,b and examples 1 and 2 page 31, paragraph 58</p> <p>---</p>	1-13
A	<p>US 3 956 491 A (ISAAC OTTO) 11 May 1976 (1976-05-11) column 5, paragraph 3</p> <p>---</p>	1-13
	-/-	

INTERNATIONAL SEARCH REPORT

International Application No	PCT/GB 00/03750
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>DATABASE WPI Section Ch, Week 199548 Derwent Publications Ltd., London, GB; Class B01, AN 1995-366914 XP002157207 & CN 1 096 031 A (UNIV SHANGHAI NO 2 MEDICAL), 7 December 1994 (1994-12-07) cited in the application abstract</p> <p>---</p>	1-13
A	<p>N YI ET AL: "Sarsasapogenin: Mechanism in treating senile dementia" SYNTHESIS AND APPLICATIONS OF ISOTOPICALLY LABELLED COMPOUNDS, XX, XX, 14 September 1997 (1997-09-14), pages 315-320, XP002117140 page 315, paragraph 2 page 319, paragraph 2</p> <p>---</p>	1-13
P, X	<p>WO 99 48482 A (HU YAER ;XIA ZONGQIN (CN); BROSTOFF JONATHAN (GB); RUBIN IAN (GB);) 30 September 1999 (1999-09-30) whole document, in particular claims 2,3 and page 11, table 2 and page 13, table 3</p> <p>-----</p>	1-13

INTERNATIONAL SEARCH REPORT

International Application No. PCT/GB 00 03750

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1,2 (in part)

The initial phase of the search revealed a very large number of documents relevant to the issue of novelty of compound claims 1 and 2. So many novelty affecting compounds (over 100) and so many documents were retrieved that it is impossible to determine which parts of the claim(s) may be said to define subject-matter for which protection might legitimately be sought (Article 6 PCT). For these reasons, a meaningful search over the whole breadth of the claim(s) is impossible. Consequently, the search on the compounds of claims 1-3 has been restricted to the most preferred compounds of dependent compound claim 3. The applicant has indeed clearly indicated on page 10, lines 22-24 of the description, that saponin derivatives of the invention "may also be commercially available", consequently by the applicant's own admission, the compounds of formula I as given in claims 1-3 are sufficiently well known in the art to be available commercially.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 00/03750

Patent document cited in search report	Publication date	Patent family member(s)			Publication date
EP 1020477	A 19-07-2000	WO 9713780	A		17-04-1997
GB 852847	A	NONE			
US 3836527	A 17-09-1974	DE 2203048	A	16-08-1973	
		AT 323344	B	10-07-1975	
		AU 465860	B	09-10-1975	
		AU 5127873	A	25-07-1974	
		BE 794362	A	23-07-1973	
		CH 578019	A	30-07-1976	
		DD 102693	A	20-12-1973	
		DK 129991	B	09-12-1974	
		ES 410828	A	16-04-1976	
		FR 2181680	A	07-12-1973	
		GB 1367025	A	18-09-1974	
		HU 164290	B	28-01-1974	
		IL 41234	A	31-05-1976	
		JP 48081847	A	01-11-1973	
		NL 7217831	A	24-07-1973	
		ZA 7209168	A	26-09-1973	
US 3929769	A 30-12-1975	CH 596238	A	15-03-1978	
		CA 1020541	A	08-11-1977	
		DD 103641	A	05-02-1974	
		DE 2324158	A	29-11-1973	
		FR 2185619	A	04-01-1974	
		GB 1427761	A	10-03-1976	
		JP 49041356	A	18-04-1974	
		NL 7306923	A	21-11-1973	
CN 1102186	A 03-05-1995	NONE			
EP 1024146	A 02-08-2000	CN 1212966	A	07-04-1999	
		AU 9336098	A	23-04-1999	
		WO 9916786	A	08-04-1999	
US 3956491	A 11-05-1976	DE 2416978	A	09-10-1975	
		AT 338968	B	26-09-1977	
		AT 266775	A	15-01-1977	
		BE 827659	A	07-10-1975	
		CA 1050427	A	13-03-1979	
		FR 2266509	A	31-10-1975	
		GB 1502873	A	08-03-1978	
		NL 7504106	A	10-10-1975	
CN 1096031	A 07-12-1994	NONE			
WO 9948482	A 30-09-1999	AU 3157499	A	18-10-1999	
		AU 3158199	A	18-10-1999	
		EP 1066042	A	10-01-2001	
		EP 1066033	A	10-01-2001	
		WO 9948507	A	30-09-1999	
		GB 2335599	A	29-09-1999	
		NO 20004804	A	09-11-2000	
		GB 2347676	A	13-09-2000	